Modified Dry Column Procedure for Extraction of Lipids from Cured Meats

MARTA P. ZUBILLAGA and GERHARD MAERKER

ABSTRACT

Polar lipids extracted from heated, nitrite-treated meats by a dry column procedure contain pigmented contaminants that interfere with subsequent analyses. Three modifications to the standard extraction procedure were studied: (a) changes in the composition of the trap, (b) insertion of additional material between column proper and trap, and (c) changes in the solvent system. Most satisfactory results were obtained by the addition of magnesium oxide to the trap (I). This resulted in the retention of impurities on the column and provided convenient, trouble-free operation. Polar lipids isolated in this manner were free of peroxides. Uncontaminated lipids were extracted from commercially processed meats by use of the modified procedure.

INTRODUCTION

IN EXPERIMENTS to develop information on the mechanism by which sodium nitrite inhibits the oxidation of meat fats during storage (Zubillaga et al., 1983) it was necessary to isolate both total lipids and the lipid subclasses from meat tissues. For this purpose a dry column extraction technique (Marmer and Maxwell, 1981) was used to isolate neutral and polar lipids separately. This technique was selected over better known ones (Folch et al., 1957; Bligh and Dyer, 1959) because of its simplicity, repeatability, and rapidity. When heated (70°C) nitrite-treated meats or commercially cured meats were extracted by this procedure, the polar lipid fractions contained polar and pigmented contaminants that interfered with the evaluation of possible antioxidant activity present in these extracts. Heme proteins and nonheme iron have been implicated as prooxidants and as promoters of warmed-over flavor in cooked meats (Love and Pearson, 1974; Igene et al., 1979; Kwoh, 1981). We have found that the pigmented contaminants of the extracted polar lipids may also exhibit prooxidant characteristics. The main objective of the present study was to modify the column extraction procedure to permit isolation of polar lipids in relatively pure form and to retard or prevent elution of interfering polar contaminants.

MATERIALS & METHODS

Materials

Celite 545 (Fisher Scientific Co.); decolorizing carbon (Norit 211, Eastman Kodak Co.); magnesium oxide (Baker Analyzed Reagents); myoglobin (Miles Laboratories Ltd.); L-α-phosphatidylcholine, dioleoyl; and L-α-phosphatidylethanolamine, dilauroyl (Sigma Chemical Co.) were used as received.

Procedures

Peroxide values. Peroxide values were determined on extracted lipids by an Official Method of the American Oil Chemists' Society (AOCS, 1979).

Thin-layer chromatography (TLC). TLC was performed on standard silica gel plates eluted with chloroform/methanol/H_2O (65/25/4).

Authors Zubillaga and Maerker are with the USDA-ARS, Eastern Regional Research Center. 600 E. Mermaid Lane, Philadelphia, PA 19118.

Fresh meat preparation. Fresh beef (bottom round) and pork (loin chops) were purchased at slaughter houses, trimmed of adipose tissue, and ground once at room temperature using a Hobart meat grinder (3/16 in. plate). To one portion of the meat (454g) a solution of 71 mg sodium nitrite in 20 ml double distilled water was added so that the final concentration of nitrite was 156 ppm. To another portion of the meat an equivalent amount of double distilled water was added for use as control. Subsequently, the nitrite-treated and control samples were treated identically. Each portion was well mixed, individually reground to assure homogeneity, and packed as 1-lb samples in plastic bags. The bags were heat-sealed under vacuum and heated to an internal temperature of 70°C during a 2-hr period. After cooling, each package was opened and the contents remixed to reincorporate juices. Portions of 25g were individually wrapped in air permeable polyvinyl chloride film and stored at 4°C.

Processed meat preparation. Commercially processed meats such as skinless poultry and beef-pork frankfurters, pepperoni, and dried beef were used in this study as purchased. However, lean portions of ham and bacon were separated from the adipose, ground in a mortar to make a homogeneous mixture, and then sampled for lipid extraction.

Standard lipid extraction procedure

Neutral and polar lipids were extracted sequentially from 5g tissue samples by a published procedure (Maxwell et al., 1980; Marmer and Maxwell, 1981).

Modified extraction procedures

Modification I. The standard extraction procedure was modified by blending magnesium oxide (0.1g) into the column trap.

Modification II. The standard extraction procedure was modified by inserting a second trap between the standard trap and the sample mixture in the column. The additional trap consisted of a uniform mixture of powdered anhydrous sodium sulfate (25g) and Celite 545 (3g). Neutral lipids were eluted as usual, but the eluate containing the polar material was collected in two portions as polar lipids (pA) and contaminants (pB). The eluate containing pA was collected until just before pigment started to emerge from the column (~110 ml). pB was then collected by further elution with approximately 25 ml of methylene chloride:methanol (9:1).

Modification III. The standard procedure was used for column preparation and extraction of neutral lipids. Polar materials were eluted in two portions (pA and pB) with a solvent mixture consisting of 7:1 chloroform:methanol which had been saturated with concentrated aqueous ammonia (about 5.5%). About 100 ml of the solvent mixture was required to elute all of the polar lipids (pA). Separation between pA and pB was based on analytical TLC.

RESULTS & DISCUSSION

HEATING of nitrite-treated pork or beef to an internal temperature of 70°C generates polar materials that contaminate the polar lipid fraction in the standard dry column elution procedure (Marmer and Maxwell, 1981). In the present work, three types of modifications of the standard procedure were studied to overcome this problem. The goal was complete elution of the polar lipid fraction free of both polar contaminants and neutral lipids. Completeness was judged by comparison with the standard elution procedure and purity by thin-layer chromatography.

The purpose of the column trap used in the standard procedure is to retain nonlipid contaminants (Maxwell et al., 1980). The active ingredient of the trap is 1g (5.8×10^{-3} mole) of $CaHPO_4 \cdot 2H_2O$. It is effective in retaining

most nonlipid impurities during the elution of lipids from raw, comminuted pork or beef muscle, but permits passage of nonlipid contaminants, especially pigmented materials, from cured meats. Several additives to the trap were examined (modification I) for their ability to enhance impurity retention. Aluminum oxide and Florisil were unacceptable, since they retained some polar lipids, especially phosphatidyl ethanolamine. Activated carbon and magnesium oxide were both satisfactory, but the latter was preferred because of convenience of handling and because its use permitted visual observation of the progress of pigments through the column and trap. Amounts of magnesium oxide added to the trap ranged from 0.1g to 1.0g per 10g of $CaHPO_4 \cdot 2H_2O$/Celite 545 (1:9); 0.1g (2.4×10^{-3} moles) was found sufficient to retain nonlipid impurities. Lesser amounts of additive might also give acceptable results but were not studied.

In another approach to the separation problem (modification II), a mixture of powdered anhydrous sodium sulfate/Celite 545 (9:1) was inserted between the column material containing the sample and the standard trap. The insert served to absorb any residual moisture remaining in the eluting polar lipid and to decrease the rate of elution of impurities (including pigments) relative to polar lipids. In contrast to modification I which retained impurities on the column, modification II permitted the separate elution of polar lipids and impurities. Separation was accomplished by visual observation of the pigmented band as it travelled through the column and by changing receivers just before the pigmented band emerged from the column. The amount of anhydrous sodium sulfate/Celite 545 mixture required to give adequate separation without retention of polar lipids may depend on the specific meat extracted. In this work use of 13g of the mixture was insufficient to give separation, while at the 33g level polar lipid recovery was somewhat decreased.

The third modification (III) involved a change in elution solvent for polar lipids. Previous authors (Rouser et al., 1963) have suggested the solvent system of chloroform: methanol (7:1) saturated with concentrated aqueous ammonia (about 5.5% v/v) for extraction of lipids free of pigmentation. In the current work methylene chloride was retained for elution of neutral lipids, but the chloroform: methanol:ammonia solvent was used in place of methylene chloride:methanol (9:1) for isolation of polar lipids. The basic solvent was effective in changing the elution pattern so that pA eluted first followed by pB.

Thin-layer chromatography was used to evaluate the effectiveness of separation of contaminants from polar lipids. Chromatography of polar lipids from beef-pork frankfurters is used for illustration (Fig. 1). The major component of pB follows phosphatidylcholine (PC) rather closely in the eluant used, but is also distinguishable from PC by its conical shape. Myoglobin remains near the origin. Comparison of the polar lipids obtained by the standard procedure and by its three modifications indicates that the cleanest product is obtained by modification I. The other two modified procedures also permit the quantitative isolation of clean, unaltered polar lipids but require close attention in the fractionation process.

Total lipid recoveries (neutral plus polar) by the standard procedure and the three modifications were compared in a

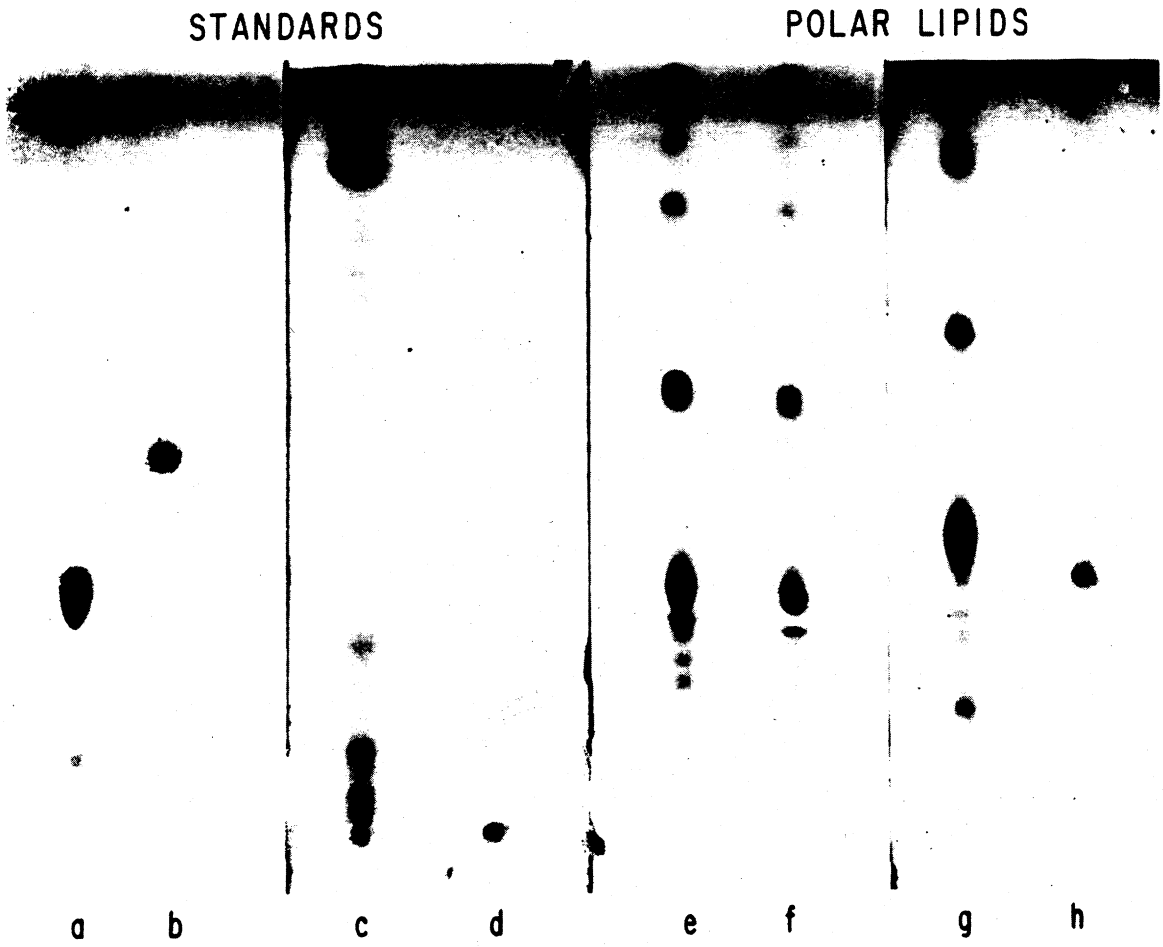

Fig. 1—Thin-layer chromatogram of standards and of polar lipids extracted from beef-pork frankfurters by several procedures: (a) phosphatidylcholine, dioleoyl; (b) phosphatidylethanolamine, dilauroyl; (c) spice mixture; (d) myoglobin. (e) standard extraction; (f) mod. I; (g) mod. II, pA; (h) mod. II, pB.

Table 1—Percent total lipids[a] in meats

Sample	Procedure			
		Modification		
	Standard	I	II	III
Beef	7.25	7.20	7.19	7.12
Beef	7.11	7.00	6.86	7.03
Beef	11.0	10.9	11.6	11.2
Beef	9.75	9.56	9.64	–
Beef	5.16	5.60	–	5.24
Pork	3.20	3.02	3.13	3.30
Pork	2.60	2.50	2.39	–

[a] Neutral plus polar fractions

set of experiments in which seven samples of ground beef and pork of varying leanness and treatment history (raw or cooked, with or without nitrite addition) were extracted in duplicate. Results are shown in Table 1 in which each number represents the average of at least two separate extractions. Evaluation of the data by a randomized block analysis of variance indicated that there was no significant ($p \leqslant 0.05$) difference in lipid recovery due to procedure, i.e., due to column composition. The data of Table 1 are sums of neutral and polar lipids isolated separately and include the lipid contaminants in all procedures except in modification I where they are retained on the column. In modifications II and III the weight of the separated pB was added to that of the polar and neutral lipids to obtain the total. In the standard procedure the contaminants were an integral part of the lipid fraction. However, since the contaminants amounted to only 3.0–11 mg in total lipids of 250–580 mg derived from 5g samples of meat, their retention on the column in modification I does not significantly lower the total amount of material isolated by that procedure.

Polar lipid fractions isolated by the three modified procedures were colorless but, those obtained by modification I appeared to be purest by TLC. The least-pure polar lipids were obtained by modification III (ammoniated elution solvent) partly because the absence of pigmented bands required separation on the basis of TLC and partly because the solvent itself was less effective in separating polar lipids from impurities.

Peroxide values of polar lipids (beef) isolated by the various procedures were measured, as were those of separated contaminant fractions (Table 2). These materials were derived from ground beef that had been heated to an internal temperature of 70°C with no added sodium nitrite. The most striking feature of this set of data is that the polar lipids obtained by modification I are entirely free of peroxides (or other substances that oxidize potassium iodide). Polar lipids from the other procedures, as well as the isolated contaminants, oxidized potassium iodide under the conditions of the peroxide test, but it was unclear if this resulted from oxidized lipids, other oxidants, or both. The pigmented contaminants are believed to be largely iron-containing derivatives of myoglobin or hemoglobin. Such compounds might be expected to give high apparent peroxide values, and in this laboratory myoglobin was found to have an apparent peroxide value of 7900.

Commercially processed meats were purchased in a local market, and their lipids were extracted both by the standard procedure and by modified methods I and II. Meats tested included chicken frankfurters, turkey frankfurters, pork and beef frankfurters, ham, bacon, dried beef, and pepperoni. Results obtained with selected commercially processed meats are summarized in Table 3. Ham and the three types of frankfurters gave reproducible lipid yields and colorless polar lipid fractions free of impurities when magnesium oxide was present in the column trap. Analysis of pepperoni presented a special problem because of the

Table 2—Peroxide values of polar fractions (beef)

Extraction procedure	Polar lipids meq/1000g	Contaminants
Standard	593	–
Modification I (MgO)	0	–
Modification II (anhyd. Na_2SO_4)	100	452
Modification III (basic solvent)	50	694

Table 3—Percent total lipids[a] in commercially processed meats

Sample	Procedures		
		Modification	
	Standard	I	II
Frankfurter (beef-pork)	28.74	28.70	28.21
Frankfurter (chicken)	19.99	19.98	19.82
Bacon	18.07	18.02	18.50
Pepperoni	42.31	40.80[b]	–
Boiled Ham	5.04	5.01	5.04

[a] Sum of neutral and polar fractions, avg of duplicate determinations.
[b] Magnesium oxide plus carbon.

presence of paprika and other spices. The highly pigmented spices contaminated both neutral and polar lipids when the standard extraction procedure was used. TLC examination (Fig. 1) of a mixture of spices such as are used in the manufacture of spiced meats demonstrated that such mixtures contain pigmented components whose R_f values approximate those of polar lipids. Reproducible extraction of clear, colorless lipid fractions from pepperoni was accomplished only by reduction of sample size from 5g to 1.2g and by use of 0.5g activated carbon, or a combination of 0.1g MgO with 0.2g carbon, in the trap. Thus, it appears that in the extraction of specific cured meats, the extraction procedure may have to be modified further to adjust for the presence of specific additives.

The work described here demonstrates that the standard dry column lipid extraction procedure requires modification for use on processed meats. All of the three modifications described can be used in the isolation of lipids that are free of pigmented impurities, but modification I is preferred because of its convenience in operation and completeness in lipid recovery.

REFERENCES

AOCS. 1979. "Official Methods and Tentative Methods," 3rd ed. American Oil Chemists' Society, Champaign, IL, Method Cd 8-53.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911.

Folch, J., Lees, M., and Sloane-Stanley, G.A. 1957. Simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497.

Igene, J.O., King, J.A., Pearson, A.M., and Gray, J.I. 1979. Influence of heme pigments, nitrite, and nonheme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agr. Food Chem. 27: 838.

Kwoh, T.L. 1981. Catalysts of lipid peroxidation in meats. J. Am. Oil Chem. Soc. 48: 550.

Love, J.D. and Pearson, A.M. 1974. Metmyoglobin and non-heme iron as prooxidants in cooked meat. J. Agr. Food Chem. 22: 1032.

Marmer, W.N. and Maxwell, R.J. 1981. Dry column method for the quantitative extraction and simultaneous class separation of lipids from muscle tissue. Lipids 16: 36.

Maxwell, R.J., Marmer, W.N., Zubillaga, M.P., and Dalikas, G.A. 1980. Determination of total fat in meat and meat products by a rapid, dry column method. J. Assoc. Off. Anal. Chem. 63(3): 600.

Rouser, G., Kritchevsky, G., Heller, D., and Lieber, E. 1963. Lipid composition of beef brain, beef liver, and the sea anemone: two approaches to quantitative fractionation of complex lipid mixtures. J. Am. Oil Chem. Soc. 40: 425.

Zubillaga, M.P., Maerker, G., and Foglia, T.A. 1983. Antioxidant activity of sodium nitrite in meat. J. Am. Oil Chem. Soc., in press.

Ms received 8/10/83; revised 10/21/83; accepted 10/24/83.

Statistical evaluation was performed by John G. Phillips. Technical assistance was rendered by C. Lyons and R. Inaker. Presented at the 42nd Annual Meeting of the Institute of Food Technologists, June 22—25, 1982, Las Vegas, NV.

Reference to brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.